PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/005462	23.12.2004	23.12.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/80, C12N15/67, C12N5/10

Applicant
DELTA BIOTECHNOLOGY LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/005462

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-75
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-75
Industrial applicability (IA)	Yes:	Claims	1-75
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V.

Reference is made to the following documents:

D1: MARTZEN MARK R ET AL: "A biochemical genomics approach for identifying genes by the activity of their products" SCIENCE (WASHINGTON D C), vol. 286, no. 5442, 5 November 1999 (1999-11-05), pages 1153-1155, XP002325596 ISSN: 0036-8075

D2: "pYEX4T-1 Vector Information" 1998, CLONTECH CATALOG #6196-1 , XP002325601

D3: PAREKH RAJESH N ET AL: "Expression level tuning for optimal heterologous protein secretion in Saccharomyces cerevisiae" BIOTECHNOLOGY PROGRESS, vol. 13, no. 2, 1997, pages 117-122, XP002325597 ISSN: 8756-7938

D4: BAO W-G ET AL: "Secretion of human proteins from yeast: stimulation by duplication of polyubiquitin and protein disulfide isomerase genes in Kluyveromyces lactis" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 272, no. 1-2, 11 July 2001 (2001-07-11), pages 103-110, XP004274844 ISSN: 0378-1119

Inventive step - Article 33(3) PCT

The application concerns expression of a target protein and a chaperone from a 2-micron plasmid. The application states that a technical prejudice in the prior art with regard to expression of proteins from 2 micron plasmids has been overcome. The application cites (pages 3-5) documents such as D3, D4 stating that expression from 2-micron constructs is less efficient than from constructs integrated on the chromosome. Said documents speculate that this is due to overloading of the secretory machinery of the cell including overloading of chaperone functions of the secretory pathway. However, the technical prejudice in the prior art is only relevant to proteins entering the secretory pathway. For proteins expressed in the cytosol, there appear to be no problems associated with the use of high copy number 2 micron plasmids. In fact, D1 shows expression on a genomewide basis of proteins from a 2 micron plasmid (see D2).

Thus, the solution proposed by the application, namely co-expression from the same plasmid of a target protein and a chaperone, is only relevant with regard to secreted proteins.

Therefore, in order for the technical effect to be recognized over the whole scope claimed, a restriction to secreted proteins is requested both for the chaperone and for the target protein (i.e. the "non-2-micron family plasmid protein"). This can be accomplished e.g. by specifying that both of said proteins comprise a signal sequence for targetting to the secretory pathway.

International Preliminary Examining Authority
European Patent Office
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24 October 2005

Sent by fax

Dear Sirs

International Patent Application No PCT/GB2004/005462
DELTA BIOTECHNOLOGY LIMITED
Our Ref: DELBE/P32303PC

This is a response to the Written Opinion of the International Searching Authority (the "ISA") dated 17 May 2005.

Inventive step

1. There is no justification for the assertion that chaperones must comprise signal sequences.

The ISA has suggested that the claims should be restricted to chaperones that possess a signal sequence.

We respectfully draw the examiner's attention to page 23, lines 5-7, which indicates that chaperones (including SSA1-4 and SSB1-2) involved in the secretory pathway may themselves be cytosolic, and therefore not all chaperones need to comprise signal sequences in order to influence the processing of secreted proteins.

We can see no justification whatsoever for the ISA's assertion that the claims should be limited to chaperones with signal sequences. With respect, this makes no technical sense. We respectfully request that this matter be favourably reconsidered.

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2. *D4 provides a prejudice in the art against the expression of cytosolic proteins from a 2μm plasmid*

The International examiner has alleged that the technical prejudice in the prior art against expressing chaperones on 2μm plasmids only applies in the case of coexpression with *secreted* heterologous proteins, citing D3 and D4. The International examiner has suggested that the cited art fails to establish a prejudice against the expression of *cytoplasmic* proteins from a multicopy plasmid. With respect, we disagree.

D4 reports that expression of the *UBI4* gene in *K. lactis* is undesirable when expressed from a multicopy plasmid and that chromosomal integration of an additional *K. lactis UBI4* gene should be chosen (see D4, page 105, section 3.1, first paragraph and section 3.2, final paragraph). *UBI4* encodes polyubiquitin, which is an intracellular protein. This is evidenced by the enclosed database entry for the *K. lactis* gene *UBI4* (accession no. Q9Y848) which reports that the "intracellular location" of the encoded protein is "nuclear and cytoplasmic" (page 2, second paragraph). Thus, D4 provides a clear prejudice against expression of cytosolic proteins from a multicopy plasmid.

Also, D3 and D4 teach that cells have thresholds beyond which the over-expression of a protein can become toxic. For example, D3 shows that secretion of the model protein BPTI saturates with increasing expression of the protein, and may actually decrease with excessively high levels of expression. It is suggested that saturation of secretion may be due to titration of available ER foldases and chaperones (page 120, first paragraph of the Discussion).

The skilled person would therefore expect that non-secreted proteins would similarly saturate cellular processes if expressed at too high a level. In fact, one may expect that over-expressed proteins that are not secreted may be more likely to become toxic (and, thus, reduce the overall cellular productivity), at high levels, compared to secreted proteins.

Since D3 and D4 teach that multi-copy plasmids such as the 2μm plasmid cause toxic levels of protein expression when *PDI1* or *UBI4* are expressed, and that this high level of toxic expression should be avoided by expression from chromosomally integrated gene copies, the skilled person would be naturally inclined to avoid the use of a multi-copy plasmid to simultaneously express a chaperone and a heterologous cytosolic protein.

cont/....

3. D1 fails to provide any motivation to deviate from the teaching of D4 and D3.

D1 was cited by the ISA as evidence that *cytosolic* proteins can be efficiently expressed from 2μm plasmids. With respect, we do not agree with this interpretation of D1, nor that the teaching of D1 can be fairly applied to that of D4 and D3.

D1 describes a biochemical genomics approach for identifying genes by the activity of their products. In D1, 6144 individual yeast strains were constructed, each carrying a plasmid, pYEX4T-1, encoding a fusion of a GST coding sequence to a different yeast ORF. The strains were pooled into 64 pools of 96 fusions, and the pools assayed for a particular biochemical activity. In active pools, the strain responsible for the biochemical activity was subsequently identified. The method therefore relies on the qualitative detection of a biochemical activity arising due to expression of a particular GST-ORF fusion in one of a bank of individual yeast strains.

The plasmid pYEX4T-1 used in D1, and further defined by D2, does not appear to have the properties of a 2μm-family plasmid. In particular, there is no indication that the plasmid encodes *FLP*, *REP1* or *REP2* which are required to maintain the plasmid at high copy number in yeast (see page 7, line 29 – page 8, line 5 of the present application. Therefore, D1 says nothing about expression of heterologous proteins from a 2μm-family plasmid.

Moreover, even if the plasmid used in D1 is a 2μm-family plasmid (for which there is no evidence), it is used in a *qualitative* screen, whereas the current application is concerned with using chaperones to effect a *quantitative* increase in protein secretion. The screening method of D1 employs positive selection, so if the effect of a particular ORF being over-expressed was that it became toxic to the host cell, it would not be distinguished from an expressed ORF which did not affect cell viability, but also had no biochemical activity. Both would be scored as negative. There is no evidence in D1 that the strains used in this study have even been tested for expression levels of the fusion proteins.

In fact, it is notable that D1's method only induces expression of the heterologous fusion protein in established cell cultures for 2 hours before the cells are harvested and the fusion proteins collected (see item 3 of the "References and Notes" on page 1155 of D1). The expression of a heterologous protein for only 2 hours, in an established cell culture, cannot fairly be considered an indication that the

cont/....

consistent over-expression of the heterologous protein is not detrimental to long term cell viability.

Accordingly, D1 says nothing to its reader about whether it is acceptable to use a gene encoded by a 2μm-family plasmid to produce large quantities of a heterologous protein (cytosolic or secreted) in a cell culture system without affecting the long-term viability and productivity of the cell culture. Therefore, D1 does not indicate that there is no difficulty in expressing cytosolic proteins from a 2μm-family plasmid, much less does it dispel the prejudice in the art (such as D3 and D4) against expression from a 2μm plasmid in order to maximise heterologous protein production.

In summary, D1 fails to provide any motivation to the skilled person, when attempting to maximise heterologous protein production, to deviate from the teaching of D4 and D3, and fails to address the prejudice in the art against the expression of *cytosolic* proteins from a 2μm plasmid.

In light of the foregoing comments, it is clear that the claims as filed possess an inventive step over the cited prior art.

Yours faithfully
ERIC POTTER CLARKSON

Richard Bassett sal/jad/ajw

Enc: Database entry for *K. lactis* UBI4